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LABORATORY INVESTIGATION

Short and long term effects of antihypertensive therapy in the diabetic rat

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Short and long term effects of antihypertensive therapy in the diabetic rat. To compare the impact of differing antihypertensive regimens on the development of renal injury, studies were performed in three groups of moderately hyperglycemic diabetic rats, and one group of non-diabetic control (C) rats. One diabetic group (DM) received no therapy except insulin. The remaining diabetic groups received insulin and either the angiotensin I converting enzyme inhibitor captopril (CAP), or triple therapy (TRX) with reserpine, hydralazine and hydrochlorothiazide. CAP and TRX modestly and comparably lowered blood pressure. At 6 to 10 weeks, DM rats exhibited elevation of the single nephron glomerular filtration rate (SNGFR), due to elevations of the glomerular capillary plasma flow rate (Q_A) and the glomerular capillary hydraulic pressure ($\bar{P}_{GC}$). In both DM/CAP and DM/TRX rats, blood pressure reduction was associated with selective normalization of $\bar{P}_{GC}$, without change in SNGFR or Q_A. In long-term (70 weeks) studies, DM rats exhibited progressive albuminuria and marked glomerular sclerosis. CAP limited albuminuria and injury to values even lower than those in C rats, whereas TRX served only to delay, but not to prevent, the increase in albuminuria. TRX reduced glomerular sclerosis, but was less effective than CAP. At 70 weeks, CAP and TRX still reduced systemic blood pressure; $\bar{P}_{GC}$ remained at normal levels with CAP but was no longer controlled with TRX. These results confirm the clinical observation that antihypertensive therapy slows diabetic glomerulopathy, but also suggest that CAP affords superior long-term protection as compared to the other antihypertensive drug regimen studied.

Microangiopathy is a major cause of morbidity and mortality in diabetes mellitus, and progressive destruction of the renal microcirculatory bed leads to end-stage renal disease in a substantial percentage of Type I and Type II diabetic patients [1]. The importance of hemodynamic factors in the progression of diabetic glomerulopathy has been documented by observations that early diabetes is characterized by elevation of the glomerular filtration rate (GFR)[1] clinically [2, 3] as well as experimentally [4–6], and that patients with the highest GFR values early in the course of the disease are more likely to progress to persistent proteinuria or overt diabetic glomerulopathy than are patients with more normal initial values for GFR [3].

Further support for a hemodynamic basis of diabetic glomerulopathy derives from studies of uninephrectomy [7, 8] or a high protein diet [5], in which increases in the glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) and perfusion and filtration rates accelerate the development of albuminuria and glomerular structural injury [5]. Conversely, interventions which attenuate these hemodynamic changes slow the development of glomerular injury. Dietary protein restriction, which lowers the single nephron (SN) GFR by reducing both the glomerular capillary plasma flow rate (Q_A) and $\bar{P}_{GC}$, affords structural protection in this model [5]. Alternatively, selective reduction of $\bar{P}_{GC}$ with the angiotensin I converting enzyme inhibitor (CEI) enalapril slows the development of injury even in the presence of continued hyperfiltration and hyperperfusion [6], suggesting that glomerular capillary hypertension is the crucial hemodynamic determinant of progressive glomerular injury. Similar findings have been documented in studies involving other models of progressive glomerular injury [9–13]. Control of glomerular hypertension may be dissociated from control of

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[1] Abbreviations used in this paper are: AI, angiotensin I; AII, angiotensin II; $\bar{A}_G$, mean glomerular cross-sectional area; $\overline{AP}$, mean arterial pressure; BG, blood glucose; C, control; CAP, captopril; CEI, converting enzyme inhibitor; DM, diabetes mellitus; GFR, glomerular filtration rate; HbA_{Ic}, glycosylated hemoglobin; Hct, hematocrit; K_f, glomerular capillary ultrafiltration coefficient; LKW, left kidney weight; PAS, periodic acid Schiff; PRA, plasma renin activity; PRC, plasma renin concentration; $\bar{P}_{GC}$, glomerular capillary hydraulic pressure; $\overline{\Delta P}$, mean glomerular transcapillary hydraulic pressure gradient; Q_A, glomerular capillary plasma flow rate; R_A, R_E, R_T, afferent, efferent and total arteriolar resistances; RAS, renin-angiotensin system; SHR, spontaneously hypertensive rat; SN, single nephron; SBP, systolic blood pressure; STZ, streptozotocin; TRX, triple therapy (reserpine, hydralazine, and hydrochlorothiazide); $U_{alb}V$, 24-hr urinary albumin excretion rate; $\bar{V}_G$, mean glomerular volume; WKY, Wistar-Kyoto rat.

systemic hypertension, in that therapy which lowers $\bar{P}_{GC}$ affords protection when systemic blood pressure is not lowered in hypertensive models [11], or only modestly reduced in normotensive animals [6, 14].

An important adverse consequence of diabetes is systemic hypertension, which contributes to acceleration of diabetic microangiopathic complications [1]. Clinical studies clearly indicate that antihypertensive therapy slows the progression of diabetic renal disease [15, 16]. However, the existing studies do not offer long-term comparisons among various antihypertensive regimens. In experimental models of non-diabetic progressive renal disease, effective antihypertensive control using the triple therapy (TRX) regimen of reserpine, hydralazine and hydrochlorothiazide reduces $\bar{P}_{GC}$ and slows the development of renal injury in some models [13, 17], whereas this regimen fails to control glomerular hypertension or to limit local injury in others [10, 18, 19]. Preliminary short-term clinical studies in non-diabetic renal disease suggest that CEI may offer a therapeutic advantage over conventional combination regimens [20–22], presumably by a selective beneficial action to reduce efferent arteriolar resistance and $\bar{P}_{GC}$, but comparison studies have not yet been performed in diabetic patients.

Accordingly, we sought to compare the early and long-term glomerular hemodynamic and structural effects of equivalent reduction in blood pressure with CEI and TRX in the moderately hyperglycemic diabetic rat. The study was specifically designed to determine the consequences of controlled reduction in blood pressure to a predetermined level, using different antihypertensive regimens, rather than to examine the effects of specific doses of pharmacologic agents. This approach, adjusting medication doses to achieve a desired level of blood pressure control, is commonly used in the clinical treatment of hypertension [15, 16, 20–22], and has revealed important differences in hemodynamic and morphologic consequences in previous studies of experimental renal disease [6, 10–14, 17–19].

Methods

Four groups of adult male Munich-Wistar rats, with initial weights of 200 to 260 g, were studied. One group (C) served as non-diabetic controls, and three groups were made diabetic by intravenous tail injection of streptozotocin (STZ, Sigma Chemical Co., St. Louis, Missouri, USA), 60 mg/kg body weight, under Brevital anesthesia (50 mg/kg i.p.). Two days later, induction of diabetes was confirmed by measurement of tail blood glucose (BG) level using a reflectance meter (Miles Ames Div., Miles Laboratories, Inc., Elkhart, Indiana, USA). Diabetic rats received daily evening injections of ultralente insulin (Iletin I, Eli Lilly & Co., Indianapolis, Indiana, USA), in doses individually adjusted to maintain BG levels between 200 and 400 mg/dl. BG levels were monitored at least once a week in all diabetic rats, and occasional values were checked in non-diabetic rats for comparison purposes. All rats were fed standard rat chow (Rodent Laboratory Chow 5001, Ralston Purina Co., Richmond, Indiana, USA) ad libitum. The diet contained the following composition by weight: protein, 23.4%; fat, 4.5%; Na, 0.40%; Ca, 1.0%; Phos, 0.61%; K, 1.10%; kcal/g, 4.25.

One diabetic group (DM) received no therapy other than insulin. A second diabetic group (DM/CAP) was treated with insulin and with the CEI captopril (E.R. Squibb & Sons, Inc., Princeton, New Jersey, USA), administered in the drinking water at a concentration of 0.75 to 1.0 g/liter. The third diabetic group (DM/TRX) received insulin and an antihypertensive triple therapy (TRX) regimen consisting of reserpine (5 mg/liter), hydralazine (160 mg/liter) and hydrochlorothiazide (25 mg/liter; Sigma Chemical Co.) in the drinking water. Previous studies have demonstrated the therapeutic efficacy of a modest reduction in systemic blood pressure with CEI therapy in this normotensive model [6]. In the present study, doses of both antihypertensive regimens were adjusted as needed to maintain the systolic blood pressure in the range of 100 to 130 mm Hg, representing a modest reduction below values seen in the DM rats receiving only insulin. Extensive pilot testing established that doses exceeding these levels resulted in excessive hypotension (systolic blood pressure < 100 mm Hg), while doses less than those reported resulted in inadequate blood pressure lowering (systolic blood pressure > 130 mm Hg). In no circumstance did a dose differing from those reported by even 25 percent result in acceptable blood pressure control.

Some of the rats in each group ($N = 8$ to 11) underwent whole kidney and glomerular hemodynamic studies at 6 to 10 weeks after STZ injection. The long-term groups were followed with monthly determinations of systolic blood pressure (SBP) by the tail cuff method [23], and were placed in metabolic cages bimonthly for determination of 24-hour urinary albumin excretion rates ($U_{alb}V$). At 70 weeks, some of the rats underwent measurement of glomerular hemodynamics, and 8 to 14 rats in each group were sacrificed for morphologic examination.

Separate groups of comparably treated rats were decapitated and trunk blood collected in EDTA after 6 to 10 weeks of diabetes for determination of plasma renin concentration (PRC).

Micropuncture studies

Following BG measurement, rats were anesthetized with Inactin (100 mg/kg i.p.) and placed on a temperature-regulated table. The left femoral artery was catheterized, and a baseline collection of blood was obtained for measurement of hematocrit (Hct), inulin "blank", and plasma sodium and potassium levels. This arterial catheter was used for subsequent periodic blood sampling and estimation of mean arterial pressure ($\overline{AP}$) via an electronic transducer connected to a direct-writing recorder. After tracheostomy, venous catheters were inserted for infusions of inulin and plasma. Intravenous infusions of rat plasma, and 10% inulin solution in 0.9% NaCl, were started at rates of 6.0 and 1.2 ml/hr, respectively. The left ureter was catheterized for urine collection, and the left kidney exposed and suspended on a lucite holder, with its surface illuminated and bathed with isotonic saline.

Since the plasma volume of rats prepared for micropuncture is reduced by ~20% [24], euvolemia was maintained using the following protocol. Isoncotic rat plasma was infused at 6 ml/hr in a total amount equal to 1% of the body weight, followed by a reduction in infusion rate to 1.6 ml/kg/hr, to maintain the Hct constant. Diabetic rats received extra saline to match the excessive urinary losses during the procedure.

For calculation of SNGFR, exactly timed samples of tubule fluid were collected for determination of flow rate and inulin concentration. Samples of efferent arteriolar blood were ob-

tained for determination of protein concentration. Coincident with these collections, arterial blood was obtained for determination of Hct and plasma concentrations of inulin and protein, and 10 to 20 minute urine collections were obtained for determination of flow rate and inulin concentration. These measurements permitted calculation of GFR (inulin clearance) by standard formulas. Time-averaged hydraulic pressures were measured in surface glomerular capillaries, proximal tubules, and efferent arterioles with a servo-null micropipette transducer system (Instrumentation for Physiology and Medicine, San Diego, California, USA). Colloid osmotic pressure of plasma entering and leaving glomerular capillaries was estimated from values for protein concentration in femoral arterial (representing afferent arteriolar) and efferent arteriolar plasma using the equation derived by Deen, et al [25]. These estimates of pre- and post-glomerular plasma protein concentration permit calculation of SN filtration fraction, glomerular capillary ultrafiltration coefficient (K_f), and afferent and efferent arteriolar blood flow rates and resistances, using equations previously described [25]. Continued hyperglycemia was confirmed by repeat BG measurement at the end of the procedure, at which time the left kidney was weighed, and blood obtained for determination of glycosylated hemoglobin (HbA_{1c}).

Morphology

Kidneys were perfused at the measured systolic arterial pressure for two to three minute with 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After perfusion fixation, the weights of both kidneys were recorded, and two midcoronal slices of 2 to 3 mm thickness were processed for light microscopic examination. Three micrometer-thick paraffin sections were stained with hematoxylin and eosin and by the periodic acid Schiff (PAS) reaction. The extent of glomerular damage was determined on PAS-stained slides by counting on two coronal sections all glomerular profiles with segmental or global collapse of capillaries with or without associated hyalin deposition and adhesion of the tuft to Bowman's capsule. On average, 472 glomeruli per animal were evaluated, and the extent of glomerular sclerosis was expressed as the percentage of glomeruli with sclerotic lesions.

The average glomerular tuft volume ($\bar{V}_G$) for each animal was determined by the procedure described by Weibel [26]. For this purpose, the mean glomerular random cross-sectional area ($\bar{A}_G$) was determined on 50 systematically sampled glomerular tuft profiles by point counting at a final magnification of 200 × using a 361-point ocular grid covering a 369,664 μm^2 microscopic field. $\bar{V}_G$ was then calculated as $\bar{V}_G = (\beta/\kappa)(\bar{A}_G)^{3/2}$, where β = 1.38 and κ = 1.1 are shape and size distribution coefficients [26, 27].

Analytical

The volume of fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The tubule fluid inulin concentration was measured by a microfluorescence method [28]. Inulin concentrations in plasma and urine were measured using a macro-anthrone method [29]. Protein concentrations were determined using a fluorometric method [30]. Urinary albumin concentration was measured by radial immunodiffusion [31]. Glycosylated hemoglobin was determined by affinity column chromatography (Glyco-Gel B, Pierce Chemical Co., Rockford, Illinois, USA) [32]. Plasma sodium and potassium levels were measured by flame photometry. PRC was determined by incubating 0.1 ml of rat plasma with 0.1 ml rat anephric plasma and 0.4 ml of 0.2 M maleate buffer, pH 6.0 at 37°C for one hour. Appropriate dilutions of rat plasma samples were made using Tris buffer. The generation of angiotensin I was then determined by radioimmunoassay using commercially available reagents (New England Nuclear, Boston, Massachusetts, USA).

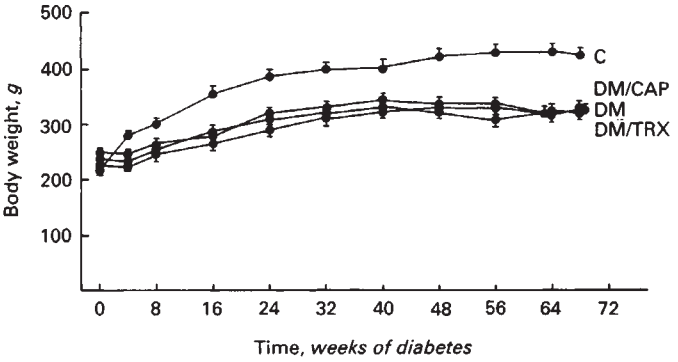

Fig. 1. *Body weight.* Serial values for body weight in non-diabetic control rats (C, N = 15), diabetic rats (DM, N = 14), and diabetic rats treated with captopril (DM/CAP, N = 10) or triple therapy (DM/TRX, N = 12). At all points after the first, all diabetic groups weighed significantly less than did C rats, but body wt values did not differ among diabetic groups. Values are means ± SEM.

Statistical

Statistical analysis was performed by one-way analysis of variance followed by computation of modified t values for six preplanned pairwise comparisons according to the method of Bonferroni [33]. Statistical significance was defined as $P < 0.05$. Values for PRC, $U_{alb}V$, and glomerular sclerosis, which were not normally distributed, were subjected to log transformation for statistical analysis. All values represent means ± SEM.

Results

General

All diabetic rats failed to gain weight during the first month of diabetes, thus resulting in slightly slower growth than in non-diabetic C rats (Fig. 1). Thereafter, rates of weight gain were fairly comparable in all groups, though body weights in the diabetic groups never reached those of C rats. Comparable hyperglycemia was maintained in all diabetic groups (Fig. 2). Values for awake systolic blood pressure (Fig. 2) were slightly elevated in DM rats, averaging 151 ± 2 mm Hg over the 70 week course, as compared to an average value of 125 ± 1 mm Hg in C rats ($P < 0.05$). In both DM/CAP (120 ± 2 mm Hg) and DM/TRX (118 ± 2 mm Hg) groups, values for SBP were modestly but significantly lower than in DM rats; at no time did values differ between CAP and TRX. Values for PRC determined after 6 to 10 weeks of diabetes are depicted in Table 1. In DM rats, values for PRC were somewhat depressed as compared to non-diabetic rats ($P < 0.05$). Inhibition of converting enzyme in DM/CAP rats was confirmed by marked elevation of PRC ($P < 0.05$ vs. all other groups). In DM/TRX rats, stimu-

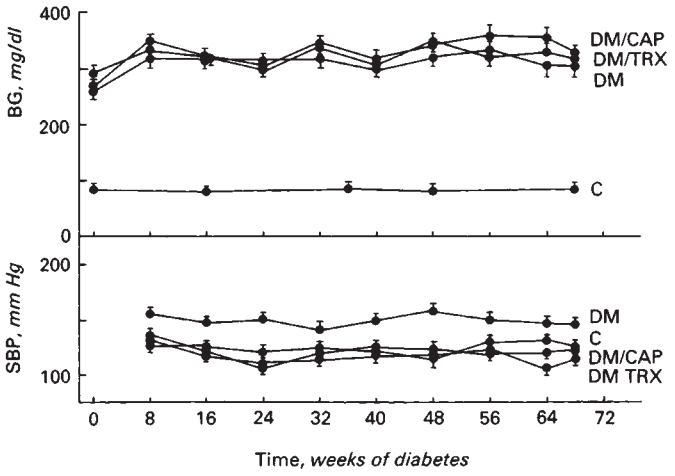

Fig. 2. *Blood glucose and systolic blood pressure.* Serial values for blood glucose (BG) and systolic blood pressure (SBP) in non-diabetic control rats (C), diabetic rats (DM), and diabetic rats treated with captopril (DM/CAP) or triple therapy (DM/TRX). All diabetic groups exhibited comparable hyperglycemia over time, with BG values consistently exceeding those in C rats, but not differing among diabetic groups. Values for SBP were modestly but significantly elevated in DM rats as compared to non-diabetic C rats at all time points, while SBP was significantly and comparably lowered in both DM/CAP and DM/TRX rats as compared to group DM. Values are means ± SEM; *N* as per Fig. 1.

lation of the plasma renin-angiotensin system was also apparent, with values for PRC above the normal range, though lower than in the DM/CAP rats ($P < 0.05$ vs. all other groups).

Insulin requirements did not differ among diabetic groups. Random measurements indicated that food and water intake were supranormal in all diabetic groups, but did not differ among them. Intakes varied from day to day in all groups; the approximate average range of daily intakes was 15 to 20 g food and 15 to 30 ml water/day for control rats, and 35 to 50 g food and 70 to 125 ml water/day for the diabetic groups.

Early micropuncture experiments

Mean values for body weight, left kidney weight (LKW), BG, HbA_{Ic}, Hct, $\overline{AP}$, whole kidney GFR, and plasma sodium and potassium levels at 6 to 10 weeks of diabetes, and in weight-matched non-diabetic control rats, are summarized in Table 2. Values for body wt did not differ among the groups. Increased kidney weight was evident in all diabetic groups, with significant and equivalent elevations of LKW as compared to C rats. Hyperglycemia, as evidenced by elevation of both BG and HbA_{Ic} levels, was present in all diabetic groups. Hematocrit was slightly lower in both DM/CAP and DM/TRX groups than in DM rats, but no diabetic group was significantly different from controls. Values for $\overline{AP}$ were equivalent in C and DM rats, whereas $\overline{AP}$ was comparably reduced in rats receiving CAP or TRX (both $P < 0.05$ vs. DM). Diabetic rats exhibited whole kidney hyperfiltration, with values in both treated groups being slightly though not significantly lower than those in DM rats, and not different from one another. Values for plasma sodium concentration were comparable in the three diabetic groups. Plasma potassium levels tended to be slightly elevated in DM/CAP rats, and were statistically lower in DM/TRX groups than in the other diabetic groups.

Table 1. Plasma renin concentration at 6 to 10 weeks of diabetes

Group	Body wt *g*	BG *mg/dl*	PRC *ng AI/ml/hr*
C ($N = 11$)	285 ± 11	89 ± 7	10.0 ± 1.5
DM ($N = 11$)	260 ± 8	286 ± 8[a]	4.9 ± 0.8[a]
DM/CAP ($N = 7$)	261 ± 6	322 ± 10[a]	353.7 ± 63.4[a,b]
DM/TRX ($N = 7$)	276 ± 12	305 ± 19[a]	46.3 ± 5.3[a,b,c]

Abbreviations are: C, non-diabetic control; DM, diabetic; DM/CAP, DM + captopril; DM/TRX, DM + triple therapy; Body wt, body weight; BG, blood glucose; PRC, plasma renin concentration; AI, angiotensin I. Values are means ± SEM.

[a] $P < 0.05$ vs. C
[b] $P < 0.05$ vs. DM
[c] $P < 0.05$ vs. DM/CAP

Mean values for SNGFR, and the pressures, flows, and resistances governing glomerular ultrafiltration are summarized in Table 3. As in previous studies [4–6], the moderately hyperglycemic diabetic rats exhibited single nephron hyperfiltration; neither CAP nor TRX affected the supranormal SNGFR values. Values for SN filtration fraction were slightly higher in DM rats, and lower in both DM/CAP and DM/TRX rats, though these differences did not reach statistical significance. All diabetic rats exhibited comparable levels of glomerular capillary hyperperfusion, with comparable elevations of Q_A as compared to controls. In DM rats, glomerular hyperperfusion was accompanied by glomerular capillary hypertension; both the glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) and the glomerular transcapillary hydraulic pressure gradient ($\overline{\Delta P}$) were elevated significantly. Diabetes induced primarily afferent arteriolar vasodilatation, so that both afferent arteriolar resistance (R_A) and the ratio of afferent arteriolar to total arteriolar resistance (R_A/R_T) were reduced. In DM/CAP and DM/TRX groups, afferent arteriolar vasodilatation was offset by concomitant efferent arteriolar vasodilatation, so that values for R_A/R_T were normalized, and values for $\bar{P}_{GC}$ and $\overline{\Delta P}$ were maintained at levels comparable to those seen in non-diabetic controls. Values for afferent and efferent protein concentrations and colloid osmotic pressures did not differ significantly. Values for K_f, the glomerular capillary ultrafiltration coefficient, were numerically though not statistically lower in the DM rats, whereas they were maintained at normal levels in both DM/CAP and DM/TRX rats; only the difference between DM and DM/TRX achieved statistical significance. Thus, all diabetic groups exhibited single nephron hyperfiltration and hyperperfusion. In the DM rats, elevated SNGFR resulted from elevations in both Q_A and $\bar{P}_{GC}$, while hyperfiltration in both treated groups resulted from elevation of Q_A and normalization of K_f. There were no significant differences between DM/CAP and DM/TRX rats in any parameter measured.

Long-term studies

As shown in Figure 2, stable moderate hyperglycemia was maintained in the three diabetic groups, and blood pressures

Table 2. Systemic and whole kidney parameters at 6 to 10 weeks of diabetes

Group	Body wt	LKW	BG	HbA_{1c}	Hct	$\overline{AP}$	GFR	Plasma Na	Plasma K
	g		*mg/dl*	*%*	*vol/dl*	*mm Hg*	*ml/min*	*mEq/liter*	
C ($N = 8$)	262 ± 8	0.95 ± 0.04	88 ± 3	5.2 ± 0.4	46 ± 1	114 ± 3	1.08 ± 0.05	—	—
DM ($N = 8$)	275 ± 7	1.21 ± 0.05[a]	286 ± 10[a]	10.7 ± 0.4[a]	48 ± 1	117 ± 3	1.69 ± 0.08[a]	139 ± 3	4.6 ± 0.3
DM/CAP ($N = 8$)	269 ± 6	1.24 ± 0.04[a]	351 ± 16[a,b]	12.8 ± 0.5[a,b]	45 ± 1[b]	98 ± 3[a,b]	1.39 ± 0.08	135 ± 3	5.3 ± 0.2
DM/TRX ($N = 11$)	270 ± 5	1.23 ± 0.05[a]	336 ± 9[a,b]	10.7 ± 0.4[a,c]	44 ± 1[b]	104 ± 3[b]	1.54 ± 0.07[a]	140 ± 2	3.4 ± 0.2[b,c]

Abbreviations are: LKW, left kidney weight; HbA_{1c}, glycosylated hemoglobin; Hct, hematocrit; $\overline{AP}$, mean arterial pressure; GFR, glomerular filtration rate. Other abbreviations as in Table 1. Values are means ± SEM. Normal values in this laboratory: Na, 143 ± 2; K, 4.0 ± 0.2.

[a] $P < 0.05$ vs. C

[b] $P < 0.05$ vs. DM

[c] $P < 0.05$ vs. DM/CAP

Table 3. Glomerular hemodynamics at 6 to 10 weeks of diabetes

Group	SNGFR	SNFF	Q_A	$\overline{P}_{GC}$	$\overline{P}_T$	$\overline{P}_E$	$\overline{\Delta P}$
	nl/min		*nl/min*	*mm Hg*			
C ($N = 8$)	42 ± 3	0.29 ± 0.02	146 ± 12	48 ± 1	14 ± 0.3	18 ± 1	34 ± 1
DM ($N = 8$)	66 ± 5[a]	0.31 ± 0.01	219 ± 19[a]	64 ± 2[a]	13 ± 0.4	18 ± 1	51 ± 2[a]
DM/CAP ($N = 8$)	61 ± 5[a]	0.27 ± 0.01	233 ± 19[a]	47 ± 1[b]	13 ± 0.4	22 ± 1	34 ± 1[b]
DM/TRX ($N = 11$)	63 ± 4[a]	0.28 ± 0.02	229 ± 14[a]	49 ± 1[b]	13 ± 1	19 ± 1	36 ± 2[b]

Abbreviations used in this table: SN, single nephron; GFR, glomerular filtration rate; FF, filtration fraction; Q_A, glomerular capillary plasma flow rate; $\overline{P}_{GC}$, mean glomerular capillary hydraulic pressure; P_T, mean proximal tubule hydraulic pressure; $\overline{P}_E$, mean efferent arteriolar hydraulic pressure; $\overline{\Delta P}$, mean glomerular transcapillary hydraulic pressure gradient; R_A, R_E and R_T, afferent, efferent, and total ($R_A + R_E$) arteriolar resistances; C_A and C_E, afferent and efferent arteriolar plasma protein concentrations; π_A and π_E, afferent and efferent arteriolar colloid osmotic pressures; K_f, glomerular capillary ultrafiltration coefficient; other abbreviations are in Table 1. Values are means ± SEM.

[a] $P < 0.05$ vs. C

[b] $P < 0.05$ vs. DM

Table 3. Continued

R_A	R_E	R_T	R_A/R_T	C_A	C_E	π_A	π_E	K_f
$\times 10^{10}$ *dyne · sec · cm*$^{-5}$				*g/dl*		*mm Hg*		*nl/(sec · mm Hg)*
1.98 ± 0.23	1.03 ± 0.06	3.01 ± 0.29	0.65 ± 0.02	5.4 ± 0.1	7.7 ± 0.2	17 ± 1	30 ± 1	0.080 ± 0.008
1.03 ± 0.08[a]	1.09 ± 0.11	2.12 ± 0.17[a]	0.49 ± 0.02[a]	5.3 ± 0.1	7.7 ± 0.2	17 ± 1	30 ± 2	0.043 ± 0.005
1.02 ± 0.07[a]	0.63 ± 0.06[a,b]	1.65 ± 0.12[a]	0.62 ± 0.01[b]	5.3 ± 0.2	7.3 ± 0.3	17 ± 1	28 ± 2	0.092 ± 0.017
1.08 ± 0.06[a]	0.71 ± 0.04[a,b]	1.79 ± 0.06[a]	0.60 ± 0.02[b]	5.4 ± 0.1	7.5 ± 0.2	18 ± 1	29 ± 1	0.113 ± 0.022[b]

were maintained slightly lower in the groups receiving antihypertensive treatment. Final determinations of SBP, body wt, LKW, BG, and HbA_{1c} are summarized in Table 4. Systolic blood pressures remained slightly elevated in DM rats, and comparably reduced in DM/CAP and DM/TRX rats. Body weights in all diabetic groups were comparable, though lower than those in C rats. Increased kidney weight was present in DM rats, but limited to intermediate values in both treated groups. Values for BG and HbA_{1c} were comparably elevated in all diabetic groups. Despite equivalent hyperglycemia and blood pressure, and comparable hemodynamics early in the course of diabetes, the long-term outcomes in the two treated groups differed substantially. Serial values for $U_{alb}V$ are depicted in Figure 3, and final values in Table 4. Non-diabetic C rats exhibited minimal $U_{alb}V$ early in life, but, as in previous studies [6, 14], developed a modest degree of age-related urinary albumin loss during the latter months of the study. Diabetic rats receiving only insulin exhibited significant elevations in $U_{alb}V$ as early as one month after induction of diabetes, and $U_{alb}V$ increased markedly over time. During the first half year of diabetes, values for $U_{alb}V$ remained in the normal range in rats receiving CAP and TRX; thereafter, values in DM/TRX rats rose substantially, eventually to values approaching those in DM rats. By the final determination, values in DM/TRX rats were still significantly lower than those in DM rats, but numerically higher than those in C rats. In contrast, after 16 months of diabetes, values in DM/CAP rats remained significantly lower than those in all other groups, including even the aging non-diabetic C rats.

Structural changes

Qualitatively, all diabetic groups exhibited identical changes in the thick ascending portion of Henle's loop and distal convoluted tubules. Areas of tubular atrophy and interstitial

Table 4. Systemic and renal parameters at 70 weeks of diabetes

	SBP	Body wt	LKW	BG	HbA_{Ic}	$U_{alb}V$	FGS[a]	$\bar{V}_G$[a]
	mm Hg	*g*		*mg/dl*	*%*	*mg/day*	*%*	*× 10^6 μm³*
C (N = 15)	126 ± 3	430 ± 6	1.99 ± 0.04	87 ± 5	5.9 ± 0.1	14 ± 3	1.4 ± 0.5	2.477 ± 0.086
DM (N = 14)	145 ± 4[b]	310 ± 11[b]	2.38 ± 0.09[b]	306 ± 17[b]	12.0 ± 0.5[b]	59 ± 8[b]	12.0 ± 2.2[b]	2.623 ± 0.074
DM/CAP (N = 10)	124 ± 5[c]	325 ± 9[b]	2.23 ± 0.14	325 ± 8[b]	11.4 ± 0.8[b]	3 ± 0.4[b,c]	0.4 ± 0.1[b,c]	2.127 ± 0.136[c]
DM/TRX (N = 12)	115 ± 4[c]	327 ± 12[b]	2.15 ± 0.08	307 ± 20[b]	10.4 ± 0.4[b,c]	36 ± 11[c,d]	4.0 ± 1.3[b,c,d]	2.074 ± 0.111[b,c]

Abbreviations are: $U_{alb}V$, 24-hr urinary albumin excretion rate; FGS, focal glomerular sclerosis; SBP, systolic blood pressure; $\bar{V}_G$, mean glomerular volume; other abbreviations as in Tables 1 and 2. Values are means ± SEM.

[a] N = 14 (C), 11 (DM), 8 (DM/CAP), 10 (DM/TRX)

[b] $P < 0.05$ vs. C

[c] $P < 0.05$ vs. DM

[d] $P < 0.05$ vs. DM/CAP

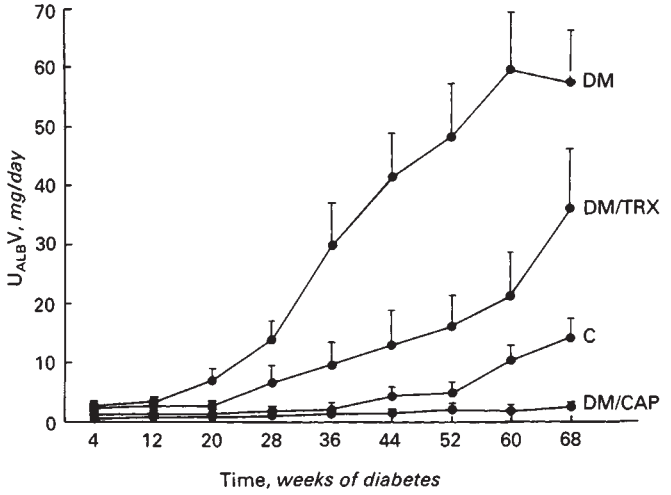

Fig. 3. *Albuminuria.* Serial values for 24-hr urinary albumin excretion ($U_{alb}V$) in non-diabetic control rats (C), diabetic rats (DM), and diabetic rats treated with captopril (DM/CAP) or triple therapy (DM/TRX). Non-diabetic C rats exhibited a slight increase in $U_{alb}V$ associated with normal aging. In DM rats, $U_{alb}V$ values were significantly higher than those in C rats at all time points. In DM/TRX rats, values for $U_{alb}V$ were near normal early in the course, but thereafter escalated; values were significantly lower than those in DM rats starting at wk 20. Values in DM/CAP rats were limited to the normal range throughout the study, being significantly lower than those in both DM and DM/TRX rats at all time points, and significantly lower than those in C rats after 60 weeks. Values are means ± SEM; N as per Fig. 1.

fibrosis were roughly proportional to the degree of glomerular involvement. Ultrastructural examination of randomly selected glomeruli (3/animal) revealed mild increases in the thickness of the glomerular basement membrane in all diabetic groups, as compared to the nondiabetic control rats. Differences in glomerular basement membrane width between diabetic groups were not readily apparent from this qualitative assessment.

Representative structural alterations in glomeruli from DM and DM/CAP rats are illustrated in Figure 4. The extent of focal and segmental glomerular sclerosis corresponded well with albuminuria values (Table 4). Non-diabetic C rats exhibited modest values for glomerular sclerosis, averaging 1.4 ± 0.5% of glomeruli (Fig. 5). The diabetic state was associated with marked glomerular injury, with sclerosis involving 12.0 ± 0.6% of glomeruli in DM rats ($P < 0.05$ vs. all other groups). As in previous studies using CEI therapy in diabetic rats [6], sclerosis was strikingly limited in DM/CAP rats, with the mean value (0.4 ± 0.1%) being significantly lower than all other groups, including the aging non-diabetic C rats. In contrast, despite equivalent blood glucose and systemic blood pressure levels, morphologic assessment in DM/TRX rats demonstrated substantially less protection. Although values for glomerular sclerosis in this group (4.0 ± 1.3%) were lower than those seen in the DM rats, they were substantially higher than those in either C or DM/CAP rats. Thus, both CAP and TRX conferred structural protection as compared to the DM rats, but the degree of protection afforded by captopril was clearly superior to that afforded by TRX.

A potential risk factor for development of glomerular sclerosis which has recently received considerable attention is increased glomerular volume [34–37]. Values for mean glomerular volume ($\bar{V}_G$) in the four groups are depicted in Table 4. Absolute values in all groups exceeded those determined in this laboratory in younger Munich-Wistar rats [34]. Values in the C and DM rats were similar. Both CAP and TRX limited glomerular volumes to values significantly lower than those in DM rats.

Late micropuncture measurements

Changes in glycemic control or systemic blood pressure control could not be invoked to explain the late development of injury in the DM/TRX rats. To further evaluate the mechanisms which might underlie this apparent late treatment failure, measurements of $\tilde{P}_{GC}$ were performed in some of the rats at the end of the 70 week study, prior to sacrifice for morphologic examination. DM/CAP rats (N = 4) exhibited continued control of glomerular hypertension, with values for $\tilde{P}_{GC}$ averaging 46 ± 1 (range, 45.0 to 47.2 mm Hg). In contrast, though studies at 6 to 10 weeks had demonstrated control of glomerular hypertension in DM/TRX rats, this effect was no longer present, with values for $\tilde{P}_{GC}$ at 70 weeks now averaging 56 ± 2 (range, 53.5 to 60.0 mm Hg, N = 4, $P < 0.002$ vs. DM/CAP). In these late studies, values for R_A were similar in the DM/CAP and DM/TRX groups, averaging 0.62 ± 0.07 and 0.95 ± 0.24 × 10^{10} dyn · sec · cm^{-5}, respectively. However, values for R_E were

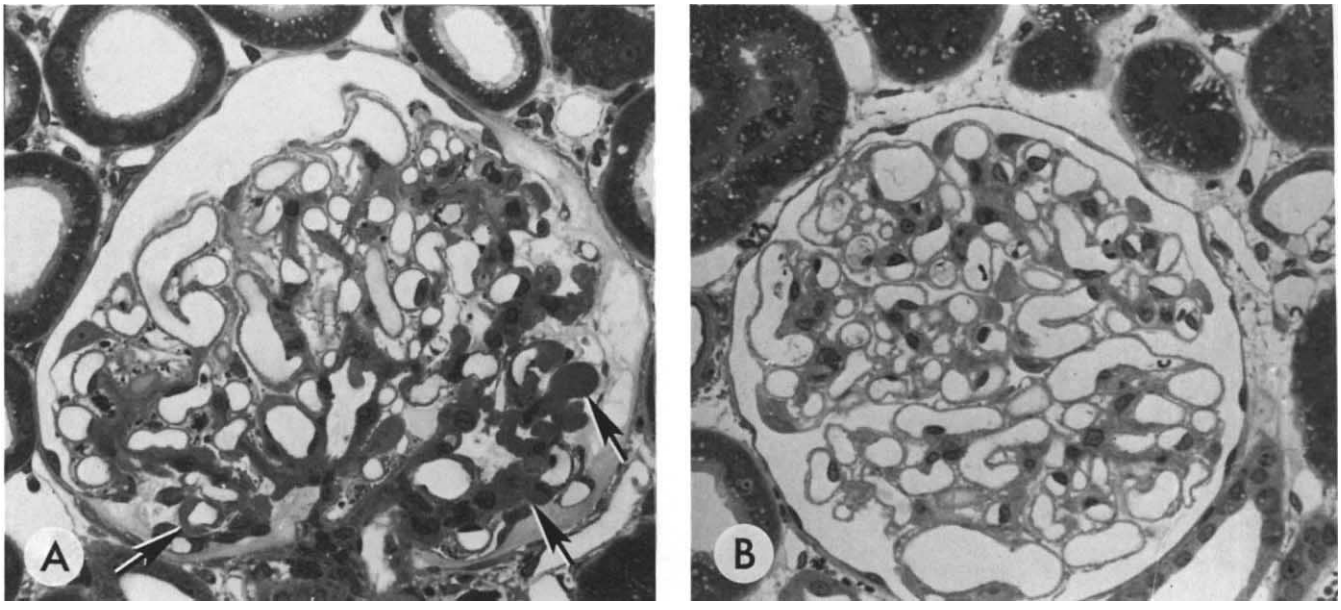

Fig. 4. *Glomerular lesions.* **(A)** Characteristic glomerular lesion from a DM rat, exhibiting segmental glomerular sclerosis and hyalin accumulation (arrows) affecting approximately 30% of the tuft area. **(B)** In comparison, the majority of glomeruli from DM/CAP rats were structurally intact. (Toluidine blue on 1 μm thick epoxy sections, × 360).

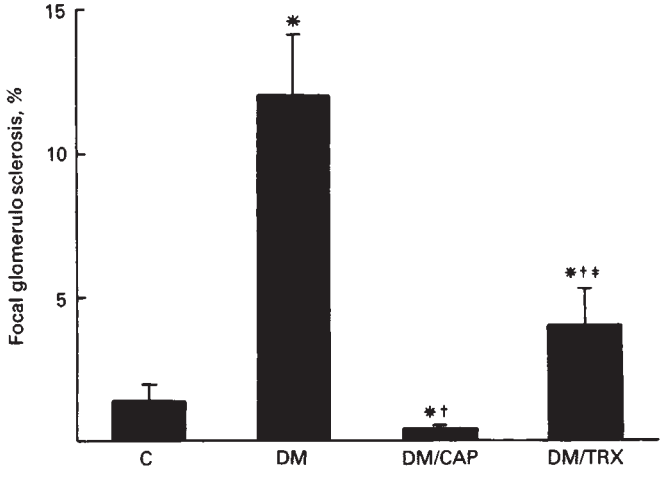

Fig. 5. *Focal glomerular sclerosis.* Mean values for focal glomerular sclerosis in non-diabetic control rats (C), diabetic rats (DM), and diabetic rats treated with captopril (DM/CAP) or triple therapy (DM/TRX). Values are means ± SEM; N as per Table 4; $^{*}P < 0.05$ vs. C, $^{\dagger}P < 0.05$ vs. DM; $^{\ddagger}P < 0.05$ vs. DM/CAP.

significantly lower in DM/CAP than in DM/TRX rats, averaging 0.40 ± 0.03 and 1.02 ± 0.12 × 10^{10} dyn · sec · cm^{-5}, respectively ($P < 0.02$). These results suggest that despite continued systemic blood pressure control with TRX, long-term TRX therapy in these rats was associated with escape from glomerular capillary hypertensive control, at a time point not directly ascertained in this study.

Discussion

In the moderately hyperglycemic normotensive diabetic rats, both monotherapy with captopril, and triple therapy with reserpine, hydralazine and hydrochlorothiazide, were effective in modestly but significantly reducing the systemic arterial pressure. Using tail cuff measurements of awake systolic blood pressure, DM rats exhibited slightly higher values than did non-diabetic controls, whereas mean arterial pressures by direct femoral artery manometry were similar in DM and C rats. The phenomenon of hypertension by tail cuff, with normotension by direct measurement, has been noted previously and attributed to stress of prewarming and restraint, as well as to reduction of skeletal muscle mass and increased fibrous tissue in the tails of streptozotocin-diabetic rats [38]. Diurnal variability in blood pressure control cannot be excluded; nevertheless, the observed blood pressure values were quite consistent in any given animal from month to month.

When studied early in the course of diabetes, the effects of captopril and TRX on glomerular hemodynamics were comparable to one another, as well as to those previously noted in diabetic rats treated with the CEI enalapril [6]. As in previous studies [4–6], the moderately hyperglycemic diabetic rats exhibited single nephron hyperfiltration and hyperperfusion. The groups treated with antihypertensive therapy exhibited lower blood pressures but similar degrees of increased kidney weight, hyperfiltration and hyperperfusion when compared to the DM rats. In all diabetic groups, values for R_A were substantially reduced as compared to the nondiabetic C rats. In the DM rats receiving no therapy other than insulin, the ratio of R_A/R_T was substantially reduced, allowing enhanced transmission of systemic pressures to the glomerular capillary network. Both CAP and TRX were associated with comparable reductions of R_A as compared to C rats; however, they also produced a concomitant reduction in R_E, which offset the reduction in R_A, thereby reducing $\bar{P}_{GC}$ to normal levels.

The effects of CAP therapy on intraglomerular hemodynamics are fully consistent with inhibition of angiotensin II-induced

enhancement of efferent arteriolar tone, as well as with numerous previous studies demonstrating efferent arteriolar relaxation with these agents in various experimental models [6, 9–14]. The intraglomerular hemodynamic consequences of TRX in this model were more difficult to predict, since TRX lowers R_E in some models [13, 17] but not in others [10, 18, 19]. Activity of the systemic blood renin-angiotensin system (RAS) does not appear to be a critical factor, since this regimen lowers R_E in some models of low renin hypertension, such as the uninephrectomized SHR [13], but not in others, including the partially nephrectomized rat [10] and mineralocorticoid-salt hypertension [18]. Of interest, this regimen raises PRC and fails to lower R_E and $\bar{P}_{GC}$ in the partially nephrectomized rat [10], which is characterized by plasma volume expansion [39]. In the moderately hyperglycemic diabetic rat, which is also characterized by plasma volume expansion [40] and low PRC values, this regimen also raises PRC, but lowers R_E and $\bar{P}_{GC}$ early in the course of diabetes. Thus, plasma volume and PRC do not appear to be consistent predictors of the glomerular hemodynamic consequences of this vasodilator/diuretic regimen. These observations are consistent with the growing body of evidence which suggests that the tissue (including intrarenal) RAS may be a more important contributor to systemic and local vascular hemodynamic regulation than is the plasma RAS [41].

During the early months of diabetes, control of systemic blood pressure with either TRX or captopril markedly limited the development of albuminuria. Thereafter, while $U_{alb}V$ values in DM/CAP rats remained at levels which were eventually even lower than those in aging non-diabetic C rats, mean values in DM/TRX rats escalated in dramatic fashion. These results are reminiscent of those previously noted in another two-kidney low-renin model, the spontaneously hypertensive rat (SHR), in which TRX was felt to prevent [42], but later found only to delay [43], the development of glomerular injury. Of particular importance is the observation that after 70 weeks of diabetes, captopril afforded continued control of glomerular capillary hypertension, as previously noted with enalapril therapy in a post-nephrotic model of glomerular injury in rats followed for a comparable period of time [14]. In contrast, despite continuing control of systemic hypertension, the aging DM/TRX rats had escaped from $\bar{P}_{GC}$ control, and exhibited a concurrent rise in albuminuria. The possibility that glomerular injury may have influenced hemodynamic findings in the DM/TRX rats studied late in the course cannot be totally excluded. However, values for $U_{alb}V$ in the animals examined were still low (< 10 mg/d) in three animals, and elevated (26 mg/day) in only one. Accordingly, it seems unlikely that glomerular injury was sufficient to explain the glomerular hypertension found in all of these animals.

As in previous studies with dietary protein restriction [5] and CEI therapy [6] in this model, development of albuminuria clearly had no relation to metabolic control; values for BG were continuously comparable in all diabetic groups, and values for HbA_{Ic} were highest in the DM/CAP rats, which consistently exhibited the lowest $U_{alb}V$ levels.

Changes in glomerular volume were also not helpful in differentiating between the outcomes of the treated groups, though the reduction in glomerular volume may help to explain the protection afforded by both regimens as compared to the DM rats. Increased glomerular volume has been noted previously in diabetes [35, 44] as well as in other experimental models characterized by hyperfiltration and particularly by glomerular hypertension [9]. When the adaptive increase in glomerular volume is prevented by dietary protein restriction in diabetic rats [35], by CEI therapy in rats with renal ablation [9], in adriamycin-induced proteinuria [34], or in a model of renal ablation with contralateral ureteral diversion [36], glomerular sclerosis is also limited. In the present study, both antihypertensive regimens limited glomerular volumes to the same extent. For the DM/CAP group, in which $\bar{P}_{GC}$ was also limited to normal values, glomerular injury was virtually non-existent. In the DM/TRX rats, lack of control of $\bar{P}_{GC}$ (at least in the late stages) appeared to be the more significant predictor of glomerular injury. Thus, changes in glomerular volume alone cannot fully explain the development of glomerular injury in this model.

The design of this study did not allow documentation of the point at which control of glomerular hypertension in DM/TRX rats was lost, though examination of the albuminuria curves may suggest that albuminuria, perhaps reflecting glomerular pressure, began to rise sharply after about six or seven months of diabetes (Fig. 3). However, evidence suggests that the levels of putative vasoactive mediators may change during the course of long-term diabetes. Early diabetes is characterized by relatively low values for plasma renin activity (PRA) and plasma AII levels, with normal levels of inactive renin and serum converting enzyme, and appropriate suppression of PRA with increased blood pressure or sodium intake [45–50]. However, later in the course, after albuminuria supervenes, values for PRA and inactive renin rise [46, 47], and converting enzyme levels tend to increase with duration of streptozotocin diabetes [51]. Lower doses of exogenous AII are required to raise blood pressure, and suppression of PRA with increased blood pressure or sodium intake is impaired [45]. These observations suggest enhanced vascular responsiveness to vasoactive stimuli, including AII, in the presence of albuminuria and diabetic glomerulopathy. In addition, it has been recently reported that the number of glomerular AII binding sites in the rat increases with advancing age [52]. In the present study, such alterations in the renin-angiotensin system over time may well have contributed to the altered glomerular hemodynamic response in the DM/TRX rats. Similarly, local prostaglandin levels and circulating levels of atrial peptides also undergo changes from normal in diabetes [40, 53] as well as during the course of the disease [53], and may also contribute to sequential changes in glomerular hemodynamics over the extended course of this study. Clearly, further studies of sequential alterations in vasoregulatory factors in diabetes are required to understand the natural history of this disease, as well as the long-term consequences of therapy.

Results in the DM/CAP rats herein confirm those noted in this laboratory with the CEI enalapril in this model [6]. In other studies in which glomerular hemodynamics were not measured, CEI therapy limited albuminuria, mesangial expansion, and glomerular basement membrane thickening in the diabetic SHR [54], and reduced blood pressure, filtration fraction (presumably reflecting $\bar{P}_{GC}$), and proteinuria in uninephrectomized diabetic rats [55]. In relatively short-term studies, administration of TRX lowered blood pressure and limited injury in the diabetic SHR [56, 57]. Bank and coworkers [57] reported that

moderately hyperglycemic diabetic SHR and Wistar Kyoto (WKY) rats both exhibited increases in SNGFR, Q_A and $\bar{P}_{GC}$ as compared to nondiabetic controls, with values for $\bar{P}_{GC}$ being slightly though significantly higher in the diabetic SHR than the diabetic WKY. Unfortunately, that study was terminated prior to the development of proteinuria or substantial glomerular injury. At the time of sacrifice, mesangial expansion was comparable in the diabetic SHR and WKY, and ameliorated in a third group of diabetic SHR in which antihypertensive therapy lowered $\bar{P}_{GC}$ toward, but not to the normal range. Thus, within the scope of that study [57], both diabetic groups exhibited glomerular hypertension and mesangial expansion, while the group receiving TRX exhibited lower values for both $\bar{P}_{GC}$ and mesangial expansion. These results are comparable to those seen in the current study, where TRX lowered $\bar{P}_{GC}$ and $U_{alb}V$ during the early months of diabetes; however, the effects of this regimen on long-term development of albuminuria and glomerular obsolescence in the diabetic SHR remain to be examined.

Clinical studies spanning as many as six years have established that antihypertensive therapy started relatively early in the course of diabetic nephropathy slows the progression of renal disease [15, 16]. Typically, the antihypertensive regimens have consisted of a diuretic, a vasodilator, and a beta-blocker, and it might be speculated that the latter would tend to suppress the RAS. However, while these studies are encouraging, they have yet to reach sufficient duration to establish whether diabetic nephropathy is truly prevented, rather than delayed. Of note, diuretic therapy (which would be expected to stimulate the RAS) has recently been correlated with accelerated development of clinical diabetic glomerulopathy [58]. In patients with advanced diabetic nephropathy, administration of captopril has been reported to markedly slow the progression of renal insufficiency [59], though only comparison to other reported studies could be made. To date there are no adequate studies comparing CEI to alternative antihypertensive regimens in diabetic patients. However, in patients with non-diabetic renal disease, several recent preliminary short-term studies suggest that CEI may be superior to conventional combination regimens in reducing proteinuria [20–22], and perhaps in slowing disease progression. That the beneficial effects of CEI are not entirely due to reduction in systemic pressure is indicated by observations that CEI therapy reduces $U_{alb}V$ in the absence of substantial changes in either blood pressure or GFR, suggesting that the reduction in $U_{alb}V$ excretion may be a direct consequence of control of $\bar{P}_{GC}$ [60–62].

In summary, the long-term consequences of captopril and TRX were compared in moderately hyperglycemic diabetic rats. Blood pressure was modestly and comparably reduced in both treated groups as compared to the normotensive diabetic rats receiving only insulin. Early in diabetes, CAP and TRX were equally effective in selectively controlling glomerular hypertension, without affecting SNGFR or Q_A. At this time, both regimens were effective in preventing albuminuria, though captopril appeared slightly more efficacious than TRX. Thereafter, TRX rats rapidly developed albuminuria, and despite continued control of systemic blood pressure, this group developed glomerular hypertension and moderate glomerular injury. Captopril therapy afforded continued control of systemic and glomerular hypertension, and prevented the development of glomerular injury. These studies lend further support to the previous observations [5, 6] that hemodynamic rather than metabolic factors predominate in the pathogenesis of glomerular injury in the diabetic rat. Furthermore, these studies confirm the clinical observations that antihypertensive therapy slows the progression of diabetic renal disease, but also indicate that captopril is more effective than TRX in preventing the development of glomerulopathy in experimental diabetes. These observations underscore the need for clinical studies to ascertain whether agents such as CEI, which offer continuing control of glomerular hypertension in the rat, will provide superior protection against the devastating glomerular and extrarenal microvascular complications of the diabetic state.

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References

1. Ritz E, Hasslacher C, Tschope W, Koch M, Mann JFE: Hypertension in diabetes mellitus. *Contrib Nephrol* 54:77–85, 1987
2. Stalder G, Schmid R: Severe functional disorders of glomerular capillaries and renal hemodynamics in treated diabetes mellitus during childhood. *Ann Paediatr* 193:129–138, 1959
3. Mogensen CE, Christensen CK: Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med* 311:89–93, 1984
4. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–415, 1981
5. Zatz R, Meyer TW, Rennke HG, Brenner BM: Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc Nat Acad Sci (USA)* 82:5963–5967, 1985
6. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM: Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 77:1925–1930, 1986
7. Steffes MW, Brown DM, Mauer SM: Diabetic glomerulopathy following unilateral nephrectomy in the rat. *Diabetes* 27:35–41, 1978
8. O'Donnell MP, Kasiske BL, Daniels FX, Keane WF: Effect of nephron loss on glomerular hemodynamics and morphology in diabetic rats. *Diabetes* 35:1011–1015, 1986
9. Anderson S, Meyer TW, Rennke HG, Brenner BM: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 76:612–619, 1985
10. Anderson S, Rennke HG, Brenner BM: Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J Clin Invest* 77:1993–2000, 1986
11. Meyer TW, Anderson S, Rennke HG, Brenner BM: Reversing glomerular hypertension stabilizes established glomerular injury. *Kidney Int* 31:752–759, 1987
12. Garcia DL, Rennke HG, Brenner BM, Anderson S: Chronic glucocorticoid therapy amplifies glomerular injury in rats with renal ablation. *J Clin Invest* 80:867–874, 1987
13. Dworkin LD, Grosser M, Feiner H, Ullian M, Randazzo J,

PARKER M: Renal vascular effects of antihypertensive therapy in the uninephrectomized SHR. *Kidney Int* 35:790–798, 1989
14. ANDERSON S, DIAMOND JR, KARNOVSKY MJ, BRENNER BM: Mechanisms underlying transition from acute glomerular injury to glomerulosclerosis in a rat model of nephrotic syndrome. *J Clin Invest* 82:1757–1768, 1988
15. MOGENSEN CE: Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *Br Med J* 285:685–688, 1982
16. PARVING H-H, ANDERSEN AR, SMIDT UM, HOMMEL E, MATHIESEN ER, SVENDSEN PA: Effect of antihypertensive treatment on kidney function in diabetic nephropathy. *Brit Med J* 294:1443–1447, 1987
17. NEUGARTEN J, KAMINETSKY B, FEINER H, SCHACHT RG, LIU DT, BALDWIN DS: Nephrotoxic serum nephritis with hypertension: Amelioration by antihypertensive therapy. *Kidney Int* 28:135–139, 1985
18. DWORKIN LD, FEINER HD, RANDAZZO J: Glomerular hypertension and injury in desoxyorticosterone-salt rats on antihypertensive therapy. *Kidney Int* 31:718–724, 1987
19. AZAR S, KEANE W, RAIJ L: Antihypertensive therapy in nephritic spontaneously hypertensive rats. Effects on nephron dynamics and morphology. (abstract) *Kidney Int* 27:187, 1985
20. HEEG JE, DE JONG E, VAN DER HEM GK, DE ZEEUW D: Reduction of proteinuria by angiotensin converting enzyme inhibition. *Kidney Int* 32:78–83, 1987
21. MANN J, RITZ E: Preservation of kidney function by use of converting enzyme inhibitors for control of hypertension. *Lancet* 2:622, 1987
22. RUILOPE LM, MIRANDA B, MORALES JM, RODICIO JL, ROMERO JC, RAIJ L: Converting enzyme inhibition in chronic renal failure. *Am J Kid Dis* 13:120–126, 1989
23. PFEFFER JM, PFEFFER MA, FROHLICH ED: Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. *J Lab Clin Med* 78:957–962, 1971
24. MADDOX DA, PRICE DC, RECTOR FC JR: Effects of surgery on plasma volume and salt and water excretion in rats. *Am J Physiol* 233:F600–F606, 1977
25. DEEN WM, TROY JL, ROBERTSON CR, BRENNER BM: Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. *J Clin Invest* 52:1500–1508, 1973
26. WEIBEL ER: *Stereological Methods: Practical Methods for Biological Morphometry*. London, Academic Press, Inc., 1979, pp. 51–57
27. HIROSE K, OSTERBY R, NOZAWA M, GUNDERSEN HJG: Development of glomerular lesions in experimental long-term diabetes in the rat. *Kidney Int* 21:689–695, 1982
28. VUREK GC, PEGRAM SE: Fluorometric method for the determination of nanogram quantities of inulin. *Anal Biochem* 16:409–419, 1966
29. FUHR J, KACMARCZYK J, KRUTTGEN CD: Eine einfache colorimetrische Methode zur Inulinbestimmung fur Nieren-clearance-Untersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin Wochenschr* 33:729–730, 1955
30. VIETS JW, DEEN WM, TROY JL, BRENNER BM: Determination of serum protein concentration in nanoliter blood samples using florescamine or o-ophthalaldehyde. *Anal Biochem* 88:513–521, 1978
31. MANCINI G, CARBONARO AO, HEREMANS JF: Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2:235–254, 1965
32. GARLICK RL, MAZER JS, HIGGINS PJ, BUNN HF: Characterization of glycated hemoglobins. Relevance to monitoring of diabetic control and analysis of other proteins. *J Clin Invest* 71:1062–1072, 1983
33. WALLENSTEIN S, ZUCKER CL, FLEISS JL: Some statistical methods useful in circulation research. *Circ Res* 47:1–9, 1980
34. FRIES JWU, SANDSTROM DJ, MEYER TW, RENNKE HG: Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. *Lab Invest* 60:205–218, 1989
35. RENNKE HG, SANDSTROM D, ZATZ R, MEYER TW, COWAN RS, BRENNER BM: The role of dietary protein in the development of glomerular structural alterations in long term experimental diabetes mellitus. (abstract) *Kidney Int* 29:289, 1986
36. YOSHIDA Y, FOGO A, ICHIKAWA I: Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int* 35:654–660, 1989
37. KLAHR S, SCHREINER GE, ICHIKAWA I: The progression of renal disease. *N Engl J Med* 318:1657–1666, 1988
38. KUSAKA M, KISHI K, SOKABE H: Does so-called streptozotocin hypertension exist in rats? *Hypertension* 10:517–521, 1987
39. KAYSEN GA, WATSON JB: Mechanism of hypoalbuminemia in the 7/8-nephrectomized rat with chronic renal failure. *Am J Physiol* 243:F372–F378, 1982
40. ORTOLA FV, BALLERMANN BJ, ANDERSON S, BRENNER BM: Atrial natriuretic peptide is a potent mediator of hyperfiltration in diabetic rats, in *Advances in Atrial Peptide Research*, edited by LARAGH JH, BRENNER BM, New York, Raven Press, 1988, pp. 350–354
41. DZAU VJ: Circulating versus local renin-angiotensin system in cardiovascular homeostasis. *Circulation* 77 (Suppl I):I-4–I-13, 1988
42. FREIS ED, RAGAN D, PILLSBURY H, MATHEWS M: Alteration of the course of hypertension in the spontaneously hypertensive rat. *Circ Res* 31:1–7, 1972
43. FELD LG, VAN LIEW JB, BRENTJENS JR, BOYLAN JW: Renal lesions and proteinuria in the spontaneously hypertensive rat made normotensive by treatment. *Kidney Int* 20:606–614, 1981
44. SEYER-HANSEN K, HANSEN J, GUNDERSEN HJG: Renal hypertrophy in experimental diabetes: A morphometric study. *Diabetologia* 18:501–505, 1980
45. WEIDMANN B, BERETTA-PICCOLI C, TROST BN: Pressor factors and responsiveness in hypertension accompanying diabetes mellitus. *Hypertension* 7 (Suppl II):II-33–II-42, 1985
46. LUETSCHER JA, KRAEMER FB, WILSON DM, SCHWARTZ HC, BRYER-ASH M: Increased plasma inactive renin in diabetes mellitus. A marker for microvascular complications. *N Engl J Med* 312:1412–1417, 1985
47. LUETSCHER JA, KRAEMER FB: Microalbuminuria and increased plasma prorenin. Prevalence in diabetics followed up to four years. *Arch Intern Med* 148:937–941, 1988
48. O'HARE JA, FERRIS JB, BRADY D, TWOMEY B, O'SULLIVAN DJ: Exchangeable sodium and renin in hypertensive diabetic patients with and without nephropathy. *Hypertension* 7 (Suppl II):II-43–II-48, 1985
49. DRURY PL, BODANSKY HJ: The relationship of the renin-angiotensin system in Type I diabetes to microvascular disease. *Hypertension* 7 (Suppl II):II-84–II-89, 1985
50. RAMOS OL: Diabetes mellitus and hypertension. State of the art lecture. *Hypertension* 11 (Suppl I):I-14–I-18, 1988
51. VALENTONOVIC MA, ELLIOTT C: Angiotensin converting enzyme activity as a function of the duration of streptozotocin-induced diabetes. (abstract) *Fed Proc* 45:463, 1986
52. WILKES BM: Increased angiotensin II receptor binding with age in isolated rat glomeruli. *Kidney Int* 34:241–247, 1988
53. CRAVEN PA, CAINES MA, DERUBERTIS FR: Sequential alterations in glomerular prostaglandin and thromboxane synthesis in diabetic rats: Relationship to the hyperfiltration of early diabetes. *Metabolism* 36:95–103, 1987
54. COOPER ME, ALLEN TJ, MACMILLAN P, BACH L, JERUMS G, DOYLE AE: Genetic hypertension accelerates nephropathy in the streptozotocin diabetic rat. *Am J Hypertens* 1:5–10, 1988
55. JACKSON B, DEBREVI L, WHITTY M, JOHNSTON CI: Progression of renal disease: effects of different classes of antihypertensive therapy. *J Hypertension* 4 (Suppl. 5):S269–S271, 1986
56. RABKIN R, PETERSEN J, KITAJI J, MARCK B, MURPHY E, MUIRHEAD EE: Effect of antihypertensive therapy on the kidney in spontaneously hypertensive rats with diabetes. (abstract) *Kidney Int* 25:205, 1984
57. BANK N, KLOSE R, AYNEDJIAN HS, NGUYEN D, SABLAY LB: Evidence against increased glomerular pressure initiating diabetic nephropathy. *Kidney Int* 31:898–905, 1987
58. WALKER WG, HERMANN J, WHELTON PK: Diuretics accelerate microalbumin excretion rates in diabetics and hypertensives. (abstract) *Kidney Int* 33:192, 1988
59. BJORCK S, NYBERG G, MULEC H, GRANERUS G, HERLITZ H, AURELL M: Beneficial effects of angiotensin converting enzyme

inhibition on renal function in patients with diabetic nephropathy. *Brit Med J* 293:471–474, 1986

60. TAGUMA Y, KITAMOTO Y, FUTAKI G, UEDA H, MONMA H, ISHIZAKI M, TAKAHASHI H, SEKINO H, SASAKI Y: Effect of captopril on heavy proteinuria in azotemic diabetics. *N Engl J Med* 313:1617–1620, 1985
61. PARVING H-H, HOMMEL E, SMIDT UM: Protection of kidney function and decrease in albuminuria by captopril in insulin dependent diabetics with nephropathy. *Brit Med J* 297:1086–1091, 1988
62. MARRE M, CHATELIER G, LEBLANC H, GUYENNE T-T, MENARD J, PASSA P: Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. *Brit Med J* 297: 1092–1095, 1988